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| L12 | l9 with L10 | 1331 | L12 |
| L11 | l9 with L10 | 1331 | L11 |
| L10 | l7 or L8 | 355526 | L10 |
| L9 | l1 or l3 or l4 | 30221 | L9 |
| L8 | pcr or (polymerase chain reaction) | 30326 | L8 |
| L7 | amplif$ | 350043 | L7 |
| L6 | l1 with L5 | 676 | L6 |
| L5 | l3 or L4 | 4822 | L5 |
| L4 | lox$ | 4822 | L4 |
| L3 | lox | 1159 | L3 |
| L2 | lox! or loxp! or loxp511! or loxa! or loxb! | 1523 | L2 |
| L1 | recombination or recombinase | 26247 | L1 |

END OF SEARCH HISTORY

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| ☑ | 5962255 | all | all | * 184 | USPT |

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09/648790
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lox P, lox P511, PER

1. Document ID: US 20020007051 A1

L1: Entry 1 of 16 File: PGPB

Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020007051
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020007051 A1

TITLE: Use of multiple recombination sites with unique specificity in recombinational cloning

PUBLICATION-DATE: January 17, 2002

US-CL-CURRENT: 536/23.1

APPL-NO: 09/ 732914
DATE FILED: December 11, 2000

RELATED-US-APPL-DATA:

| RLAN | RLFD | RLPC | RLKC | RLAC |
|---|---|---|---|---|
| 60169983 | Dec 10, 1999 | | | US |
| 60188020 | Mar 9, 2000 | | | US |

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of the filing dates of U.S. application Ser. No. 60/169,983, filed Dec. 10, 1999, and U.S. application Ser. No. 60/188,020, filed Mar. 9, 2000, both of which are incorporated herein by reference.

IN: Cheo, David, Brasch, Michael A., Temple, Gary F., Hartley, James L., Byrd, Devon R. N.

AB: The present invention provides compositions and methods for recombinational cloning. The compositions include vectors having multiple recombination sites with unique specificity. The methods permit the simultaneous cloning of two or more different nucleic acid molecules. In some embodiments the molecules are fused together while in other embodiments the molecules are inserted into distinct sites in a vector. The invention also generally provides for linking or joining through recombination a number of molecules and/or compounds (e.g., chemical compounds, drugs, proteins or peptides, lipids, nucleic acids, carbohydrates, etc.) which may be the same or different. Such molecules and/or compounds or combinations of such molecules and/or compounds can also be bound through recombination to various structures or supports according to the invention.

L1: Entry 1 of 16 File: PGPB

Jan 17, 2002

DOCUMENT-IDENTIFIER: US 20020007051 A1
TITLE: Use of multiple recombination sites with unique specificity in recombinational cloning

Summary of Invention Paragraph (41):
[0042] Mutating specific residues in the core region of the att site can generate a large number of different att sites. As with the att1 and att2 sites utilized in GATEWAY.TM., each additional mutation potentially creates a novel att site with unique specificity that will recombine only with its cognate partner att site bearing the same mutation and will not cross-react with any other mutant or wild-type att site. Novel mutated att sites (e.g., attB 1-10, attP 1-10, attR 1-10 and attL 1-10) are described in previous patent application Ser. No. 09/517,466, filed Mar. 2, 2000, which is specifically incorporated herein by reference. Other recombination sites having unique specificity (i.e., a first site will recombine with its corresponding site and will not recombine or not substantially recombine with a second site having a different specificity) may be used to practice the present invention. Examples of suitable recombination sites include, but are not limited to, loxP sites; loxP site mutants, variants or derivatives such as loxP511 (see U.S. Pat. No. 5,851,808);frt sites; frt site mutants, variants or derivatives; difsites; difsite mutants, variants or derivatives; psi sites; psi site mutants, variants or derivatives; cer sites; and cer site mutants, variants or derivatives. The present invention provides novel methods using such recombination sites to join or link multiple nucleic acid molecules or segments and more specifically to clone such multiple segments (e.g., two, three, four, five, seven, ten, twelve, fifteen, twenty, thirty, fifty, seventy-five, one hundred, two hundred, etc.) into one or more vectors (e.g., two, three, four, five, seven, ten, twelve, etc.) containing one or more recombination sites (e.g., two, three, four, five, seven, ten, twelve, fifteen, twenty, thirty, fifty, seventy-five, one hundred, two hundred, etc.), such as any GATEWAY.TM. Vector including Destination Vectors.

Detail Description Paragraph (57):
[0295] Recombination sites for use in the invention may be any nucleic acid that can serve as a substrate in a recombination reaction. Such recombination sites may be wild-type or naturally occurring recombination sites, or modified, variant, derivative, or mutant recombination sites. Examples of recombination sites for use in the invention include, but are not limited to, phage-lambda recombination sites (such as attP, attB, attL, and attR and mutants or derivatives thereof) and recombination sites from other bacteriophage such as phi80, P22, P2, 186, P4 and P1 (including lox sites such as loxP and loxP511). Mutated att sites (e.g., attB 1-10, attP 1-10, attR 1-10 and attL 1-10) are described in Example 9 below and in previous patent U.S. application Ser. No. 60/136,744, filed May 28,1999, and U.S. application Ser. No. 09/517,466, filed Mar. 2, 2000, which are specifically incorporated herein by reference. Other recombination sites having unique specificity (i.e., a first site will recombine with its corresponding site and will not recombine with a second site having a different specificity) are known to those skilled in the art and may be used to practice the present invention. Corresponding recombination proteins for these systems may be used in accordance with the invention with the indicated recombination sites. Other systems providing recombination sites and recombination proteins for use in the invention include the FLP/FRT system from Saccharomyces cerevisiae, the resolvase family (e.g., .gamma..delta., TndX, TnpX, Tn3 resolvase, Hin, Hjc, Gin, SpCCE1, ParA, and Cin), and IS231 and other Bacillus thuringiensis transposable elements. Other suitable recombination systems for use in the present invention include the XerC and XerD recombinases and the psi, dif and cer recombination sites in E. coli. Other suitable recombination sites may be found in U.S. Pat. No. 5,851,808 issued to Elledge and Liu which is specifically incorporated herein by reference. Preferred recombination proteins and mutant, modified, variant, or derivative recombination sites for use in the invention include those described in U.S. Pat. Nos. 5,888,732 and 6,143,557, and in U.S. application Ser. No. 09/438,358 (filed Nov. 12, 1999), based upon United States provisional application Ser. No. 60/108,324 (filed Nov. 13, 1998), and U.S. application Ser. No. 09/517,466 (filed Mar. 2, 2000), based upon U.S. provisional application Ser. No. 60/136,744 (filed May 28, 1999), as well as those associated with the GATEWAY.TM. Cloning Technology available from Invitrogen Corp., Life Technologies Division (Rockville, Md.), the entire disclosures of all of which are specifically incorporated herein by reference in their entireties.

2. Document ID: US 20010021769 A1

L1: Entry 2 of 16 File: PGPB

Sep 13, 2001

PGPUB-DOCUMENT-NUMBER: 20010021769
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010021769 A1

TITLE: Somatic cells with ablated PrP gene and methods of use

PUBLICATION-DATE: September 13, 2001

US-CL-CURRENT: 530/388.8; 435/7.23, 435/70.21

APPL-NO: 09/ 829507
DATE FILED: April 9, 2001

RELATED-US-APPL-DATA:

| | | | |
|---|---|---|---|
| RLAN | RLFD | RLPC | RLKC |
| RLAC | | | |
| 09829507 | Apr 9, 2001 | ABANDONED | A1 |
| US | | | |
| 09220265 | Dec 22, 1998 | GRANTED | |
| US | | | |
| 09220265 | Dec 22, 1998 | | |
| US | | | |
| 08740947 | Nov 5, 1996 | | |
| US | | | |
| 5834593 | | | |

CROSS-REFERENCE

[0001] This application is a continuation-in-part application of Ser. No. 08/740,947, filed Nov. 5, 1996, which is incorporated herein by reference in its entirety and to which application we claim priority under 35 USC .sctn.120.

IN: Prusiner, Stanley B.

AB: The present invention comprises a method for producing mammalian therapeutics free from prion contamination and cells for use in such methods. Such therapeutics are produced in somatic cells having a genome with an artificially altered PrP gene. The PrP gene in these cells may be ablated, or replaced by an exogenous inducible form of the PrP gene. The endogenous gene in the host cells may be disrupted, or disrupted and replaced by an exogenous PrP gene.

L1: Entry 2 of 16 File: PGPB

Sep 13, 2001

DOCUMENT-IDENTIFIER: US 20010021769 A1
TITLE: Somatic cells with ablated PrP gene and methods of use

Detail Description Paragraph (15):
[0083] The site-specific recombination-facilitating sequences useful in the present invention may be either a naturally-occurring sequence or a modified sequence. For example, PCT published application no. WO 93/19172 describes phage vectors in which the VH10 genes are flanked by two loxP sites, one of which is a mutant loxP site (loxP511), in which the G at the seventh position in the spacer region of loxP is replaced with an A, preventing recombination within the vector from merely excising the V.sub.H genes. However, two loxP511 sites can recombine via Cre-mediated recombination and, therefore, can be recombined selectively in the presence of one or more wild-type lox sites. The nucleotide sequences of the insert repeats and the spacer region of loxP511 are as follows:

3. Document ID: US 20010008026 A1

L1: Entry 3 of 16 File: PGPB

Jul 12, 2001

PGPUB-DOCUMENT-NUMBER: 20010008026
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010008026 A1

TITLE: SYSTEM FOR TISSUE-RESTRICTED GENE RECOMBINATION

PUBLICATION-DATE: July 12, 2001

US-CL-CURRENT: 800/8; 435/325, 435/455, 435/462, 435/463, 435/465, 800/14, 800/18, 800/21, 800/22, 800/25, 800/3, 800/9

APPL-NO: 09/ 104654
DATE FILED: June 25, 1998
CONTINUED PROSECUTION APPLICATION: CPA
IN: SCHNEIDER, MICHAEL D., OVERBEEK, PAUL, FRENKEL, PETER

AB: The present invention relates to methods and compositions for tissue-restricted gene recombination. In particular, the present invention provides methods and compositions for tissue-restricted gene recombination in post-mitotic cells. The present invention further provides methods for gene recombination in post-mitotic cells comprising the delivery of a Cre recombinase to the target tissue to facilitate recombination in a desired target nucleic acid.

L1: Entry 3 of 16 File: PGPB

Jul 12, 2001

DOCUMENT-IDENTIFIER: US 20010008026 A1
TITLE: SYSTEM FOR TISSUE-RESTRICTED GENE RECOMBINATION

Summary of Invention Paragraph (10):
[0011] In certain embodiments of the present invention, the post-mitotic target tissue comprises cardiac tissue, although the methods of the present invention are applicable to any post-mitotic target

tissue. In other embodiments, the target nucleic acid
comprises a gene. In yet other embodiments, the one or more site-specific
recombination target sequences comprises one or more
loxP target sequences, although other site-specific recombination target
sequences are contemplated by the present invention,
including, but not limited to, loxP2, loxP3, loxP23, loxP511, loxB, loxC2,
loxL, loxR, lox.DELTA.86, lox.DELTA.117, frt, dif, flp,
and att target sequences.

Detail Description Paragraph (107):
[0139] The examples provided above utilize loxP recombination target
sequences. However, the present invention is not limited to
the use of these particular target sequences. A variety of additional
recombination target sites are known in the art including,
but not limited to, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR,
loxA86, lox.DELTA.117, frt, dif, flp and att, which
will find use with the present invention.

4. Document ID: US 6261808 B1

L1: Entry 4 of 16

File: USPT

Jul 17, 2001

US-PAT-NO: 6261808
DOCUMENT-IDENTIFIER: US 6261808 B1

TITLE: Amplification of nucleic acid molecules via circular replicons

DATE-ISSUED: July 17, 2001

US-CL-CURRENT: 435/91.1; 435/91.2, 536/23.1

APPL-NO: 9/ 657943
DATE FILED: September 8, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. patent application Ser. No.
09/188,214 (filed Nov. 9, 1998), which application
is incorporated herein by reference in its entirety, and which is a continuation
application of U.S. patent application Ser. No.
08/906,491 (filed Aug. 5, 1997, which issued on Nov. 10, 1998, as U.S. Pat.
No. 5,834,202), which application is a
continuation-in-part of U.S. patent application Ser. No. 08/595,226 (filed Feb.
1, 1996; issued Mar. 31, 1998, as U.S. Pat. No.
5,733,733), which is a continuation-in-part of U.S. patent application Ser. No.
08/533,852 (filed Sep. 26, 1995; issued Mar. 25,
1997, as U.S. Pat. No. 5,614,389), which is a continuation-in-part of U.S.
patent application Ser. No. 08/383,327 (filed Feb. 3,
1995; issued Jan. 7, 1997, as U.S. Pat. No. 5,591,609), which is a
continuation-in-part of PCT Application No. PCT/US93/07309
(filed Aug. 4, 1993), which is a continuation-in-part of U.S. patent application
Ser. No. 07/933,945, filed Aug. 24, 1992 (which
application was abandoned in favor of continuation application U.S. patent
application Ser. No. 08/136,405, filed Oct. 15, 1993,
which issued on Oct. 11, 1994 as U.S. Pat. No. 5,354,668), which is a
continuation-in-part of U.S. patent application Ser. No.
07/924,643, filed Aug. 4, 1992 (now abandoned).
IN: Auerbach; Jeffrey I.

AB: Methods and compositions suitable for accomplishing the in
vitro amplification of nucleic acid molecules
via enzymatic means are provided. The preferred means employ circular
rather than linear replicons. Means for
producing such circular replicons from linear reactants are also provided.

L1: Entry 4 of 16

File: USPT

Jul 17, 2001

DOCUMENT-IDENTIFIER: US 6261808 B1
TITLE: Amplification of nucleic acid molecules via circular replicons

Detailed Description Paragraph Right (21):
Experiments with mutant lox sites in which either the left or right inverted
repeat had been removed, has revealed that Cre is
capable of binding to partial loxP sites, but is incapable of mediating efficient
recombination between such sites. Insertions in
the spacer region impair the ability of Cre to catalyze recombination. Of
particular interest to the present invention is the use
of a loxP511 mutant site.

5. Document ID: US 6255071 B1

L1: Entry 5 of 16

File: USPT

Jul 3, 2001

US-PAT-NO: 6255071
DOCUMENT-IDENTIFIER: US 6255071 B1

TITLE: Mammalian viral vectors and their uses

DATE-ISSUED: July 3, 2001

US-CL-CURRENT: 435/69.1; 435/320.1, 435/455, 435/6, 536/23.1,
536/23.5, 536/24.1

APPL-NO: 8/ 820931
DATE FILED: March 19, 1997

PARENT-CASE:

This application is a Continuation-In-Part of application Ser. No. 08/716,926,
filed Sept. 20, 1996 and now U.S. Pat. No.
6,025,192 which is incorporated herein by reference in its entirety.
IN: Beach; David H., Hannon; Gregory J., Conklin; Douglas, Sun;
Peiqing

AB: The present invention relates to methods and compositions for
the elucidation of mammalian gene function.
Specifically, the present invention relates to methods and compositions
for improved mammalian complementation
screening, functional inactivation of specific essential or non-essential
mammalian genes, and identification of
mammalian genes which are modulated in response to specific stimuli.,
In particular, the compositions of the present
invention include, but are not limited to, replication-deficient retroviral
vectors, libraries comprising such
vectors, retroviral particles produced by such vectors in conjunction with
retroviral packaging cell lines,
integrated provirus sequences derived from the retroviral particles of the
invention and circularized provirus
sequences which have been excised from the integrated provirus
sequences of the invention. The compositions of the
present invention further include novel retroviral packaging cell lines.

L1: Entry 5 of 16

File: USPT

Jul 3, 2001

DOCUMENT-IDENTIFIER: US 6255071 B1
TITLE: Mammalian viral vectors and their uses

Detailed Description Paragraph Right (82):
The retroviral and pEHRE vectors displaying random peptide sequences of the present invention can comprise, (a) a splice donor site or a LoxP site (e.g., LoxP511 site); (b) a bacterial promoter (e.g., pTac) and a shine-delgarno sequence; (c) a pel B secretion signal for targeting fusion peptides to the periplasm; (d) a splice-acceptor site or another LoxP511 site (Lox P511 sites will recombine with each other, but not with the LoxP site in the 3' LTR); (e) a peptide display cassette or vehicle; (f) an amber stop codon; (g) the M13 bacteriophage gene 111 protein C-terminus (amino acids 198-406); and optionally the vector may also comprise a flexible polyglycine linker.

6. Document ID: US 6218152 B1

L1: Entry 6 of 16 File: USPT

Apr 17, 2001

US-PAT-NO: 6218152
DOCUMENT-IDENTIFIER: US 6218152 B1

TITLE: In vitro amplification of nucleic acid molecules via circular replicons

DATE-ISSUED: April 17, 2001

US-CL-CURRENT: 435/91.2; 435/91.1, 536/23.1

APPL-NO: 9/ 188214
DATE FILED: November 9, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation application of U.S. patent application Ser. No. 08/906,491 (filed Aug. 5, 1997), which issued on Nov. 10, 1998, as U.S. Pat. No. 5,834,202, which application is incorporated herein by reference in its entirety, and which application is a continuation-in-part of U.S. patent application Ser. No. 08/595,226 (filed Feb. 1, 1996; issued Mar. 31, 1998, as U.S. Pat. No. 5,733,733), which is a continuation-in-part of U.S. patent application Ser. No. 08/533,852 (filed Sep. 26, 1995; issued Mar. 25, 1997, as U.S. Pat. No. 5,614,389), which is a continuation-in-part of U.S. patent application Ser. No. 08/383,327 (filed Feb. 3, 1995; issued Jan. 7, 1997, as U.S. Pat. No. 5,591,609), which is a continuation-in-part of PCT Application No. PCT/US93/07309 (filed Aug. 4, 1993), which is a continuation-in-part of U.S. patent application Ser. No. 07/933,945, filed Aug. 24, 1992 (which application was abandoned in favor of continuation application U.S. patent application Ser. No. 08/136,405, filed Oct. 15, 1993, which issued on Oct. 11, 1994 as U.S. Pat. No. 5,354,668), which is a continuation-in-part of U.S. patent application Ser. No. 07/924,643, filed Aug. 4, 1992 (now abandoned).
IN: Auerbach; Jeffrey I.

AB: Methods and compositions suitable for accomplishing the in vitro amplification of nucleic acid molecules
via enzymatic means are provided. The preferred means employ circular rather than linear replicons. Means for producing such circular replicons from linear reactants are also provided.

L1: Entry 6 of 16 File: USPT

Apr 17, 2001

DOCUMENT-IDENTIFIER: US 6218152 B1
TITLE: In vitro amplification of nucleic acid molecules via circular replicons

Detailed Description Paragraph Right (20):
Experiments with mutant lox sites in which either the left or right inverted repeat had been removed, has revealed that Cre is capable of binding to partial loxP sites, but is incapable of mediating efficient recombination between such sites. Insertions in the spacer region impair the ability of Cre to catalyze recombination. Of particular interest to the present invention is the use of a loxP511 mutant site.

7. Document ID: US 6025192 A

L1: Entry 7 of 16 File: USPT

Feb 15, 2000

US-PAT-NO: 6025192
DOCUMENT-IDENTIFIER: US 6025192 A

TITLE: Modified retroviral vectors

DATE-ISSUED: February 15, 2000

US-CL-CURRENT: 435/320.1; 435/6, 435/DIG.24, 536/23.1, 536/23.5, 536/24.1

APPL-NO: 8/ 716926
DATE FILED: September 20, 1996
IN: Beach; David, Hannon; Gregory J.

AB: The present invention relates to methods and compositions for the elucidation of mammalian gene function.
Specifically, the present invention relates to methods and compositions for improved mammalian complementation screening, functional inactivation of specific essential or non-essential mammalian genes, and identification of mammalian genes which are modulated in response to specific stimuli., In particular, the compositions of the present invention include, but are not limited to, replication-deficient retroviral vectors, libraries comprising such vectors, retroviral particles produced by such vectors in conjunction with retroviral packaging cell lines, integrated provirus sequences derived from the retroviral particles of the invention and circularized provirus sequences which have been excised from the integrated provirus sequences of the invention. The compositions of the present invention further include novel retroviral packaging cell lines.

L1: Entry 7 of 16 File: USPT

Feb 15, 2000

DOCUMENT-IDENTIFIER: US 6025192 A
TITLE: Modified retroviral vectors

Detailed Description Paragraph Right (36):
The vectors displaying random peptide sequences of the present invention can comprise, (a) a splice donor site or a LoxP site (e.g., LoxP511 site); (b) a bacterial promoter (e.g., pTac) and a shine-delgarno sequence; (c) a pel B secretion signal for targeting fusion peptides to the periplasm; (d) a splice-acceptor site or another LoxP511 site (LoxP511 sites will recombine with each other, but not with the LoxP site in the 3' LTR); (e) a peptide display cassette or vehicle; (f) an amber stop codon; (g) the M13 bacteriophage gene 111 protein C-terminus (amino acids 198-406); and optionally the vector may also comprise a flexible polyglycine linker.

8. Document ID: US 6010884 A

L1: Entry 8 of 16

File: USPT

Jan 4, 2000

US-PAT-NO: 6010884
DOCUMENT-IDENTIFIER: US 6010884 A

TITLE: Recombinant binding proteins and peptides

DATE-ISSUED: January 4, 2000

US-CL-CURRENT: 435/69.7; 435/252.3, 435/320.1, 435/471, 536/23.4

APPL-NO: 8/ 654623
DATE FILED: May 29, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This is a continuation of International Application No. PCT/GB94/02662, filed Dec. 5, 1994, and a continuation-in-part of U.S. Ser. No. 08/448,418, filed May 14, 1996, now U.S. Pat. No. 5,837,242, and claims priority for earlier application PCT/GB94/02662.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| GB | 9225453 | December 4, 1992 |
| GB | 9300816 | January 16, 1993 |
| EP | 93303614 | May 10, 1993 |
| GB | 9319969 | September 22, 1993 |
| GB | 9412147 | June 17, 1994 |

IN: Griffiths; Andrew David, Holliger; Kaspar Philipp, Nissim; Ahuva, Fisch; Igor, Winter; Gregory Paul

AB: DNA constructs comprise a first exon sequence of nucleotides encoding a first peptide or polypeptide, a second exon sequence of nucleotides encoding a second peptide or polypeptide and a third sequence of nucleotides between the first and second sequences encoding a heterologous intron, for example that of Tetrahymena thermophila nuclear pre-rRNA, between RNA splice sites and a site-specific recombination sequence, such as loxP, within the intron, the exons together encoding a product peptide or polypeptide. Such constructs are of use in methods of production of peptides or polypeptides, transcription leading to splicing out of the intron enabling translation of a single chain product peptide or polypeptide. Isolated nucleic acid constructs consisting essentially of a sequence of nucleotides encoding a self-splicing intron with a site-specific recombination sequence within the intron, for use in creation of constructs for expression of peptides or polypeptides, are also provided.

L1: Entry 8 of 16

File: USPT

Jan 4, 2000

DOCUMENT-IDENTIFIER: US 6010884 A
TITLE: Recombinant binding proteins and peptides

Brief Summary Paragraph Right (35):
A way of enriching for productive recombination events is to employ mutant sites. Several mutants of the lox P sequence are known, and these are compromised with respect to their ability to recombine with each other and the wild-type lox P sequence (Hoess, R. H., Wierzbicki, A. and Abremski, K. (1986) Nucl. Acids Res. 14, 2287-2300). For example, lox P 511 SEQ ID NO:5 has a G->A point mutation in the central 8 bp segment, with the result that it will only recombine with other lox P 511 SEQ ID NO:5 sites, but not the wild-type lox P sequence (Hoess, R. H. Wierzbicki, A. and Abremski, K. (1986) et supra.). Placement of wild-type and mutant lox P sequence combinations can direct which recombination events are possible. The sites loxP1, loxP2, loxP3 and loxP4 (FIG. 4; SEQ ID NOS: 7, 8, 6, & 9, respectively) can be used in a similar way to loxP511 SEQ ID NO:5. These sites do not recombine significantly with loxP511. There is in some cases a degree of recombination between the loxPWT site and these mutant sites, derived from it. For instance, in one experiment 5% recombination was observed between loxP3 SEQ ID NO:6 and loxPWT sites. All of these new loxP sites recombine efficiently with identical sites, ie like sites, eg one loxP4 site with another loxP4 site, and show strong preference for this over recombination with a different site.

Brief Summary Paragraph Right (38):
For example, a clone specific for an antigen may be isolated where the gene for a VH domain of a scFv fragment is located between loxP511 and loxp wt SEQ ID NO:4 of a vector containing 3 loxP sites, such as fd3lox. A library of VL domains may then be shuffled with the VH domain gene kept constant by recombining the clon in the 3 loxP site vector with a library of VL genes on a donor vector such as pUC19 which are located between the loxP4 site and the loxp 511 SEQ ID NO:5 site. The library of VL domain genes is now encoded in the 3 lox site vector and scFv fragments, eg with improved affinity, may be selected from the phage displayed scFv fragment repertoire.

Brief Summary Paragraph Right (42):
The use of three different loxP sites also allows, for example, the recombination of three sequences in order. One sequence to be recombined could be flanked by loxP and loxP511 SEQ ID NO:5, a second sequence by loxP511 SEQ ID NO:5 and loxP3SEQ ID NO:6. These sequences may then be recombined into a third replicon containing a third DNA sequence and three loxP sites. The location of 2 loxP sites within different self splicing introns allows the three sequences to be expressed continuously as shown in FIGS. 7 and 8.

Brief Summary Paragraph Right (87):
FIG. 13a shows the fd phage acceptor vector, fdDWT/4 containing 3 lox sites

is shown. It contains the VH and VL genes of the anti-NIP clone G6 (Griffiths et al, 1994 supra). The sites loxP511 and loxPWT SEQ ID NO:4 flank the VH gene and the sites loxPWT and loxP4 SEQ ID NO:9 flank the VL gene. The loxPWT site is in the self splicing intron and the loxP4 site sits between the VL gene and gene III. The diabody or single chain Fv polypeptide chain encoded is expressed as a fusion with the gene III protein. A site for the factor X protease is included between the VL gene and gene III to allow the possibility of the elution by proteolysis of phage from the antigen during selection procedures. Alternative versions of fdDWT/4 were also made with the site loxP4 replaced with loxP3 SEQ ID NO:6 and loxP1 SEQ ID NO:7 respectively. The donor vector PDN8 contains the VH-D10 gene flanked by loxP511 and loxPWT sites. The donor vector pRWT/4 contains the VL-D10 gene flanked by loxPWT SEQ ID NO:4 and loxP4 sites. In the donor vectors pRWT/3 or pWT/1 the loxP4 SEQ ID NO:9 site of pRWT/4 is replaced by the loxP3 SEQ ID NO:6 or loxP1 SEQ ID NO:7 site respectively. The expression vector pEX511/4 contains the S12 gene, which confers streptomycin sensitivity on bacteria, flanked by loxP511 and loxP4 sites.

Brief Summary Paragraph Right (88):
FIG. 13B summarises the recombination efficiencies obtained in the experiments described in example 6. The left hand loxP site is loxP511, the middle loxP site is the loxP site within the self splicing intron and the right hand loxP site is the loxP site between the VL gene and gene III .

Detailed Description Paragraph Right (58):
The fd phage acceptor vector, fdDWT/4 containing 3 lox sites is shown in FIG. 13. It contains the VH and VL genes of the anti-NIP clone G6 (Griffiths et al, 1994 supra). The sites loxP511 SEQ ID NO:5 and loxPWT SEQ ID NO:4 flank the VH gene and the sites loxPWT and loxP4 SEQ ID NO:9 flank the VL gene. The loxPWT site is in the self splicing intron and the loxP4 site sits between the VL gene and gene III. The diabody or single chain Fv polypeptide chain encoded is expressed as a fusion with the gene III protein. A site for the factor X protease is included between the VL gene and gene III to allow the possibility of the elution by proteolysis of phage from antigen during selection procedures. Alternative versions of fdDWT/4 were also made with the site loxP4 replaced with loxP3 SEQ ID NO:6 and loxP1 SEQ ID NO:7 respectively (see FIG. 12).

Detailed Description Paragraph Right (59):
If, for example, a VL gene repertoire is first cloned into fdDWT/4 as ApaLI-AscI fragments, a VH gene repertoire may then be introduced by recombination with a donor vector containing the VH gene repertoire, flanked by loxP511 SEQ ID NO:5 and loxPWT sites.

Detailed Description Paragraph Right (60):
fSeWT/4 was recombine d with the donor vector pDN8 containing the VH-D10 gene flanked by loxP511 and loxPWT sites. This was performed by transforming E. coli TGI pACYCaraCre (Example 5) with pND8 donor vector containing VH-D10 and then infecting with fdWT/4 phage containing the genes encoding the variable domains, VH-GG and VL-GG. Recombination was allowed to continue at 30.degree. C. overnight. Recombined phage from the bacterial supernatant were used to infect TG-1. As a result of recombination between the loxP511 sites of donor and acceptor and between the loxPWT sites of the donor and acceptor, the recombined fd phage contains VH-D10 while keeping the original VL-G6.

Detailed Description Paragraph Right (66):
To test the feasibility of subcloning directly into an expression vector by recombination using the loxP sites, the expression vector pEX511/4 was constructed (FIG. 13). This contains the S12 gene, which confers streptomycin sensitivity on bacteria, flanked by loxP511 SEQ ID NO:5 and loxP4 SEQ ID NO:9 sites. E. coli TG1 pACYCaraCre (Example 5) were transformed with pEX511/4 and then infected with fdDST/4 containing the genes encoding the variable domains, VH-G6 and VL-G6.

9. Document ID: US 6010861 A

L1: Entry 9 of 16 File: USPT

Jan 4, 2000

US-PAT-NO: 6010861
DOCUMENT-IDENTIFIER: US 6010861 A

TITLE: Target specific screens and their use for discovering small organic molecular pharmacophores

DATE-ISSUED: January 4, 2000

US-CL-CURRENT: 435/7.1; 435/6, 435/69.1, 436/536

APPL-NO: 8/ 473105
DATE FILED: June 7, 1995

PARENT-CASE:

This is a continuation-in-part of abandoned U.S. application Ser. No. 08/286,084 filed Aug. 3, 1994 and which is incorporated in its entirety herein.

IN: Blume; Arthur J.

AB: The invention relates to a general process by which recombinantly derived variable domains of antibodies encompassing either or both light and heavy variable regions with or without respective constant regions are engineered and selected for identification of unique surface domains of pharmaceutical targets or parts thereof which regulate target function. The recombinant antibodies are useful as reagents for high volume, rapid screening of occupation of the active surface domains by natural or synthetic entities. This invention is also directed to elucidating the three dimensional conformation of the antibodies, or parts thereof, which bind to the pharmaceutical targets and confers activity. Methods for creating high resolution molecular models which can direct the synthesis of biologically active small organic molecules useful as viable discovery drug leads are also provided.

L1: Entry 9 of 16 File: USPT

Jan 4, 2000

DOCUMENT-IDENTIFIER: US 6010861 A
TITLE: Target specific screens and their use for discovering small organic molecular pharmacophores

Detailed Description Paragraph Right (245):
Construction is begun by reamplification of the rVHCH1 library maintained in the pVHACCEPTOR.lib.bact. using PCR, as described above, with primers pCFWD and pCBCK. The DNA product is isolated and cut with VHrs2' (Nco1) and VHrs4 (Not1) and is ligated using T4 ligase and standard methodology into LoxPRO precut with Nco1 and Not1. The LoxPRO used in this example is fashioned after the pUC based plasmid as described by Griffiths, A. D. et al. 1994) and contains an endogenous CH1, bounded by a Sfil and Not1 rs, preceded by a ribosome binding site (rbs), an in frame LpelB leader sequence (LpelB), followed by an inframe wild type loxP sequence (Hoess et al. 1982) and then an inframe myc sequence. In LoxPRO upstream from the LpelB is a mutant loxP511 sequence. DNA from the ligation mixture is purified and electroporated (Dower, Miller et al. 1988) into E. coli TG1 (Gibson 1984) to create the pUC based library LoxPRO.rVHCH1lib. (i.e., pUCLoxPROVIDER-rVHCH1lib). More than 10.sup.8 clones are obtained

and the diversity is
confirmed by sequencing independent clones.

10. Document ID: US 5851808 A

L1: Entry 10 of 16 File: USPT

Dec 22, 1998

US-PAT-NO: 5851808
DOCUMENT-IDENTIFIER: US 5851808 A

TITLE: Rapid subcloning using site-specific recombination

DATE-ISSUED: December 22, 1998

US-CL-CURRENT: 435/91.4; 435/320.1, 435/91.41, 536/23.1

APPL-NO: 8/ 864224
DATE FILED: February 28, 1997
IN: Elledge; Stephen J., Liu; Qinghua

AB: The present invention provides compositions, including vectors, and methods for the rapid subcloning of nucleic acid sequences in vivo and in vitro. In particular, the invention provides vectors used to contain a gene of interest that comprise a sequence-specific recombinase target site. These vectors are used to rapidly transfer the gene of interest into any expression vector that contains a sequence-specific recombinase target site located downstream of a promoter element so that the gene of interest may be expressed.

L1: Entry 10 of 16 File: USPT

Dec 22, 1998

DOCUMENT-IDENTIFIER: US 5851808 A
TITLE: Rapid subcloning using site-specific recombination

Brief Summary Paragraph Right (6):
The present invention is not limited by the nature of the sequence-specific recombinase target site employed on the nucleic acid construct. In one embodiment, the sequence-specific recombinase target site is selected from the group consisting of loxP, loP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, lox.DELTA.86, lox.DELTA.117,frt, dif, and att.

Brief Summary Paragraph Right (10):
The present invention is not limited by the nature of the sequence-specific recombinase target site employed on the nucleic acid construct. In one embodiment, the sequence-specific recombinase target site is selected from the group consisting of loxP, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, lox.DELTA.86, lox.DELTA.117,frt, dif, and att.

Brief Summary Paragraph Right (13):
In another preferred embodiment, the nucleic acid construct further comprises a selectable marker gene. The present invention is not limited by the nature of the selectable marker gene chosen; the selectable marker may be a positive or negative selectable marker. In a preferred embodiment, the selectable marker is selected from the group consisting of the kanamycin resistance gene, the ampicillin resistance gene, the tetracycline resistance gene, the chloramphenicol resistance gene, the streptomycin resistance gene, the strA gene and the sacB gene. The present invention is not limited by the nature of the sequence-specific recombinase target site employed on the nucleic acid construct. In one embodiment, the sequence-specific recombinase target site is selected from the group consisting of loxP, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, lox.DELTA.86, lox.DELTA.117, frt, dif and att.

Detailed Description Paragraph Right (6):
The term "lox site" as used herein refers to a nucleotide sequence at which the product of the cre gene of bacteriophage Pl, Cre recombinase, can catalyze a site-specific recombination. A variety of lox sites are known to the art including the naturally occurring loxP (the sequence found in the Pl genome), loxB, loxL and loxR (these are found in the E. coli chromosome) as well as a number of mutant or variant lox sites such as loxP511, lox.DELTA.86, lox.DELTA.117, loxC2, loxP2, loxP3 and loxP23.

Detailed Description Paragraph Right (60):
The Cre protein also recognizes a number of variant or mutant lox sites (variant relative to the loxP sequence), including the loxB, loxL and loxR sites which are found in the E. coli chromosome [Hoess et al. (1982), supra]. Other variant lox sites include loxP511 [5'-ATAACTTCGTATAGTATACATTATACGAAGTTAT-3' (SEQ ID NO:16); spacer region underlined; Hoess et al. (1986), supra], loxC2 [5'-ACAAC TTCGTATAATGTATGCTATACGAAGTTAT-3' (SEQ ID NO:17); spacer region underlined; U.S. Pat. No. 4,959,317). Cre catalyzes the cleavage of the lox site within the spacer region and creates a six base-pair staggered cut [Hoess and Abremski (1985) J. Mol. Biol. 181:351]. The two 13 bp inverted repeat domains of the lox site represent binding sites for the Cre protein. If two lox sites differ in their spacer regions in such a manner that the overhanging ends of the cleaved DNA cannot reanneal with one another, Cre cannot efficiently catalyze a recombination event using the two different lox sites. For example, it has been reported that Cre cannot recombine (at least not efficiently) a loxP site and a loxP511 site; these two lox sites differ in the spacer region. Two lox sites which differ due to variations in the binding sites (ie., the 13 bp inverted repeats) may be recombined by Cre provided that Cre can bind to each of the variant binding sites; the efficiency of the reaction between two different lox sites (varying in the binding sites) may be less efficient that between two lox sites having the same sequence (the efficiency will depend on the degree and the location of the variations in the binding sites). For example, the loxC2 site can be efficiently recombined with the loxP site; these two lox sites differ by a single nucleotide in the left binding site.

Detailed Description Paragraph Right (131):
As described in Example 6, cotransformation of E. coli cells expressing Cre protein (e.g., QLB4, BNN132) with a pUNI construct and a pHOST construct (each construct containing a single lox site) results in the fusion of these two constructs in vivo. If the host cell used for the recombination reaction constitutively expresses the Cre protein, multimeric forms of the fused constructs are generated. In addition to the methods outlined above for tightly regulating the expression of the cre gene in the host cell, cells constitutively producing Cre protein can be employed with modified pUNI and pHOST vectors as described in this example. The pUM construct is modified such that two different lox sites flank the kanamycin resistance gene (the modified pUNI construct is termed pUNI-D). The two lox sites differ in their spacer regions by one or two nucleotides and for the sake of discussion the two different lox sites are referred to as "loxA" and "loxB" (e.g., loxP and loxP511; "loxb" is used in this discussion to distinguish it from the first lox site termed "loxA" and does not indicate the use of the loxB sequence found in the E. coli chromosome). Cre cannot efficiently catalyze a recombination event between a loxA site and a loxb due to the sequence changes located in the spacer regions between the Cre binding sites; however Cre can efficiently catalyze the recombination between two loxA sites or two loxB sites [Hoess et al. (1986) Nucleic Acids Res.14:2287]. The pHOST construct is modified such that one loxA site and one loxB site flank the selectable marker gene (the modified pHOST construct is termed pHOST-D). In this example, pHOST contains the sacB gene

as the selectable marker (a negative selectable marker). The presence of the sacB gene on pHOST-D provides a means of
counter-selection as cells expressing the sacB gene are killed when the cell is grown in medium containing 5% sucrose [Gay et al.
(1985) J. Bacteriol. 164:918 and (1983) J. Bacteriol. 153:1424].

Detailed Description Paragraph Right (135):
pUNI-10 was modified to place a second lox site, comprising the loxP511 sequence (SEQ ID NO:16) between the kanamycin resistance
gene and the R6K.gamma. conditional origin of replication to create pUNI-10-D. A second lox site, comprising the loxP511 site, was
inserted onto a loxP-containing expression plasmid (i.e., a pHOST vector) to create a pHOST-D vector. One-half of one microgram of
each plasmid was cotransformed into competent QLB4 cells and an aliquot of the transformed cells were plated onto LB/Ap plates and
onto LB/Ap/Kn plates containing 5% sucrose and the number of colonies on each type of plate were counted. The percentage of
AP.RTM.Kn.RTM. colonies which grew on sucrose-containing plates relative to the number of Ap.RTM. colonies was 1%
(1.times.10.sup.3 /1.times.10.sup.5). Restriction enzyme digestion of plasmid DNA isolated from individual Ap.RTM.KW.RTM. colonies
which grew on sucrose-containing plates confirmed that the desired fusions had been generated. These results indicate that the in
vivo gene trap method can be used to recombine a gene of interest carried on a pUNI-D vector into an expression vector using host
cells that constiutively express the Cre protein.

Detailed Description Paragraph Right (136):
In addition to providing a means for recombining a gene of interest carried on a pUNI-D vector into an expression vector using
host cells that constitutively express the Cre protein, the in vivo gene trap method provides a means to transfer a gene of
interest contained on a linear DNA molecule (e.g., a PCR product) that lacks a selectable marker into an expression vector(s). The
desired PCR product is amplified using two primers, each of which encode a different lox site (a "loxA" and "loxB" site such as a
loxP and loxP511 site). A pUNI vector is constructed that contains (5' to 3') a loxA site, a counter-selectable marker such as the
sacB gene and a lox B site (i.e., the two different lox sites flank the counter-selectable marker). This pUNI vector also contains
a conditional origin of replication and an antibiotic resistance gene as described above and in Ex. 1. The PCR product
(loxA-amplified sequence-loxB) is recombined with the modified pUNI vector (which comprises loxA-counter-selectable marker-loxB)
to create a pUNI vector containing the PCR product which now lacks the counter-selectable marker; this recombination event is
selected for by growing the host cells in medium which kills the host if the counter-selectable gene is expressed. The PCR product
in the pUNI vector (containing 2 lox sites) can then be placed under the control of the desired promoter element by recombining
the pUNI/PCR product construct with the appropriate pHOST-D vector.

CLAIMS:

8. The vector construct of claim 1, wherein said sequence-specific recombinase target site is selected from the group consisting
of loxP, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, lox.DELTA.86, lox.DELTA.117, frt, dif, and att.

11. Document ID: US 5834202 A

L1: Entry 11 of 16 File: USPT

Nov 10, 1998

US-PAT-NO: 5834202
DOCUMENT-IDENTIFIER: US 5834202 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: November 10, 1998

US-CL-CURRENT: 435/6; 435/320.1, 435/91.1, 435/91.2, 536/23.1, 536/24.2, 536/24.33

APPL-NO: 8/ 906491
DATE FILED: August 5, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. patent application Ser. No. 08/595,226 (filed Feb. 1, 1996, which issued on
Mar. 31, 1998, as U.S. Pat. No. 5,733,733) which is a continuation-in-part of U.S. patent application Ser. No. 08/533,852 (filed
Sep. 26, 1995, which issued on Mar. 25, 1997, as U.S. Pat. No. 5,614,389) which is a continuation-in-part of U.S. patent
application Ser. No. 08/383,327 (filed Feb. 3, 1995, which issued on Jan. 7, 1997, as U.S. Pat. No. 5,591,609), which is a
continuation-in-part of PCT Application No. PCT/US93/07309 (filed Aug. 4, 1993), which is a continuation-in-part of U.S. patent
application Ser. No. 07/933,945, filed Aug. 24, 1992 (which application was abandoned in favor of continuation application U.S.
patent application Ser. No. 08/136,405, filed Oct. 15, 1993, which issued on Oct. 11, 1994 as U.S. Pat. No. 5,354,668), which is a
continuation-in-part of U.S. patent application Ser. No. 07/924,643, filed Aug. 4, 1992 (now abandoned).

IN: Auerbach; Jeffrey I.

AB: Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the
amplification is performed under isothermal conditions. The invention also includes kits containing reagents for
conducting the method.

L1: Entry 11 of 16 File: USPT

Nov 10, 1998

DOCUMENT-IDENTIFIER: US 5834202 A
TITLE: Methods for the isothermal amplification of nucleic acid molecules

Detailed Description Paragraph Right (20):
Experiments with mutant lox sites in which either the left or right inverted repeat had been removed, has revealed that Cre is
capable of binding to partial loxP sites, but is incapable of mediating efficient recombination between such sites. Insertions in
the spacer region impair the ability of Cre to catalyze recombination. Of particular interest to the present invention is the use
of a loxP511 mutant site.

12. Document ID: US 5733733 A

L1: Entry 12 of 16 File: USPT

Mar 31, 1998

US-PAT-NO: 5733733
DOCUMENT-IDENTIFIER: US 5733733 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: March 31, 1998

US-CL-CURRENT: 435/6; 435/320.1, 435/5, 435/91.1, 435/91.2, 536/23.1, 536/24.3, 536/24.33

APPL-NO: 8/ 595226
DATE FILED: February 1, 1996

PARENT-CASE:

FIELD OF THE INVENTION
The present invention is in the field of recombinant DNA technology. This invention is directed to a process for amplifying a
nucleic acid molecule, and to the molecules employed and produced through this process.
CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. patent application Ser. No. 08/533,852 (filed Sep. 26, 1995, now U.S. Pat. No.
5,614,389) which is a continuation-in-part of U.S. patent application Ser. No. 08/383,327 (filed Feb. 3, 1995) now U.S. Pat. No.
5,591,609, which is a continuation-in-part of PCT Application No. PCT/US93/07309 (filed Aug. 4, 1993), which is a
continuation-in-part of U.S. patent application Ser. No. 07/933,945, filed Aug. 24, 1992 (which application was abandoned in favor
of continuation application U.S. patent application Ser. No. 08/136,405, filed Oct. 15, 1993, which issued on Oct. 11, 1994 as
U.S. Pat. No. 5,354,668), which is a continuation-in-part of U.S. patent application Ser. No. 07/924,643, filed Aug. 4, 1992 (now
abandoned).
IN: Auerbach; Jeffrey I.

AB: Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the
amplification is performed under isothermal conditions. The invention also includes kits containing reagents for
conducting the method.

L1: Entry 12 of 16 File: USPT

Mar 31, 1998

DOCUMENT-IDENTIFIER: US 5733733 A
TITLE: Methods for the isothermal amplification of nucleic acid molecules

Detailed Description Paragraph Right (20):
Experiments with mutant lox sites in which either the left or right inverted repeat had been removed, has revealed that Cre is
capable of binding to partial loxP sites, but is incapable of mediating efficient recombination between such sites. Insertions in
the spacer region impair the ability of Cre to catalyze recombination. Of particular interest to the present invention is the use
of a loxP511 mutant site.

13. Document ID: US 5614389 A

L1: Entry 13 of 16 File: USPT

Mar 25, 1997

US-PAT-NO: 5614389
DOCUMENT-IDENTIFIER: US 5614389 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: March 25, 1997

US-CL-CURRENT: 435/91.2; 435/6, 435/91.1

APPL-NO: 8/ 533852
DATE FILED: September 26, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. patent application Ser. No. 08/383,327 (filed Feb. 3, 1995), which is a
continuation-in-part of PCT Application No. PCT/US93/07309 (filed Aug. 4, 1993), which is a continuation-in-part of U.S. patent
application Ser. No. 07/933,945, filed Aug. 24, 1992 (which application was abandoned in favor of continuation application U.S.
patent application Ser. No. 08/136,405, filed Oct. 15, 1993, which issued on Oct. 11, 1994 as U.S. Pat. No. 5,354,668, U.S. patent
application Ser. No. 07/933,445, which is a continuation-in-part of U.S. patent application Ser. No. 07/924,643, filed Aug. 4,
1992 (now abandoned).
IN: Auerbach; Jeffrey I.

AB: Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the
amplification is performed under isothermal conditions. The invention also includes kits containing reagents for
conducting the method.

L1: Entry 13 of 16 File: USPT

Mar 25, 1997

DOCUMENT-IDENTIFIER: US 5614389 A
TITLE: Methods for the isothermal amplification of nucleic acid molecules

Detailed Description Paragraph Right (20):
Experiments with mutant lox sites in which either the left or right inverted repeat had been removed, has revealed that Cre is
capable of binding to partial loxP sites, but is incapable of mediating efficient recombination between such sites. Insertions in
the spacer region impair the ability of Cre to catalyze recombination. Of particular interest to the present invention is the use
of a loxP511 mutant site.

14. Document ID: US 5591609 A

L1: Entry 14 of 16 File: USPT

Jan 7, 1997

US-PAT-NO: 5591609
DOCUMENT-IDENTIFIER: US 5591609 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: January 7, 1997

US-CL-CURRENT: 435/91.2; 435/6, 435/91.1, 435/91.5

APPL-NO: 8/ 383327
DATE FILED: February 3, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of PCT Application No. PCT/US93/07309 (filed Aug. 4, 1993), which is a continuation-in-part of U.S. patent application Ser. No. 07/933,945, filed Aug. 24, 1992 (which application was abandoned in favor of continuation application U.S. patent application Ser. No. 08/136,405, filed Oct. 15, 1993, which issued on Oct. 11, 1994 as U.S. Pat. No. 5,354,668) and U.S. patent application Ser. No. 07/933,945 is a continuation-in-part of U.S. patent application Ser. No. 07/924,643, filed Aug. 4, 1992 (now abandoned).
IN: Auerbach; Jeffrey I.

AB: Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the amplification is performed under isothermal conditions. The invention also includes kits containing reagents for conducting the method.

L1: Entry 14 of 16 File: USPT Jan 7, 1997

DOCUMENT-IDENTIFIER: US 5591609 A
TITLE: Methods for the isothermal amplification of nucleic acid molecules

Detailed Description Paragraph Right (19):
Experiments with mutant loxP sites in which either the left or right inverted repeat had been removed, has revealed that Cre is capable of binding to partial loxP sites, but is incapable of mediating efficient recombination between such sites. Insertions in the spacer region impair the ability of Cre to catalyze recombination. Of particular interest to the present invention is the use of a loxP511 mutant site.

AB: Methods and recombinant vectors suitable for accomplishing the in vivo alteration of a nucleic acid molecule are disclosed. The invention in particular discloses the use of recombinases such as Cre to accomplish in vivo recombination.

L1: Entry 15 of 16 File: USPT Jul 18, 1995

DOCUMENT-IDENTIFIER: US 5434066 A
TITLE: Modulation of CRE recombinase in the in vivo cloning of DNA

Brief Summary Paragraph Right (37):
In one embodiment, the Cre protein may be the expression product of a mutated cre gene, and may interact with mutated loxP sites. Suitable mutations have been produced both in Cre, and in the loxP site. The Cre mutants thus far identified have been found to catalyze recombination at a much slower rate than that of the wild-type Cre protein. loxP mutants (such as loxP511) have been identified which recombine at lower efficiency than the wild-type site (Abremski, K., et al., J. Biol, chem, 261:391-396 (1986); Abremski, K., et al., J. Mol. Biol. 202:59-66 (1988), herein incorporated by reference).

Brief Summary Paragraph Right (57):
In a similar manner, vectors containing other preselected genes, such as the Cre gene, can be employed. For example, the host cell can be constructed to contain and express the .lambda.int gene (the gene may be present on the target molecule, the nucleic acid molecule that contains the preselected gene, or any other nucleic acid molecule (including the cell's chromosome)), and the desired preselected gene (for example, cre) can be present on a nucleic acid molecule flanked by the attP and attB sites, as described above. In lieu of the .lambda.int gene, other recombinases (such as mutant Cre proteins) or other recombinogenic sites such as loxP511 can be employed.

15. Document ID: US 5434066 A

L1: Entry 15 of 16 File: USPT Jul 18, 1995

US-PAT-NO: 5434066
DOCUMENT-IDENTIFIER: US 5434066 A

TITLE: Modulation of CRE recombinase in the in vivo cloning of DNA

DATE-ISSUED: July 18, 1995

US-CL-CURRENT: 435/475; 435/252.3, 435/252.33, 435/476

APPL-NO: 8/ 214023
DATE FILED: March 15, 1994

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation application of U.S. Patent application Ser. No. 07/862,188 filed Apr. 2, 1992 now abandoned which is a continuation-in-part of U.S. Patent application Ser. No. 07/825,267 filed Jan. 24, 1992, now abandoned.
IN: Bebee; Robert L., Hartley; James L.

16. Document ID: US 5354668 A

L1: Entry 16 of 16 File: USPT Oct 11, 1994

US-PAT-NO: 5354668
DOCUMENT-IDENTIFIER: US 5354668 A

TITLE: Methods for the isothermal amplification of nucleic acid molecules

DATE-ISSUED: October 11, 1994

US-CL-CURRENT: 435/91.1; 435/6

APPL-NO: 8/ 136405
DATE FILED: October 15, 1993

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS
This application is a continuation of U.S. patent application Ser. No. 07/933,945, filed Aug. 24, 1992, now abandoned, which application was a continuation-in-part of U.S. patent application Ser. No. 07/924,643, filed Aug. 4, 1992.
IN: Auerbach; Jeffrey I.

AB: Methods for amplifying a nucleic acid molecule which employs a single primer, and in which the
amplification is performed under isothermal conditions. The invention also includes kits containing reagents for
conducting the method.

L1: Entry 16 of 16

File: USPT

Oct 11, 1994

DOCUMENT-IDENTIFIER: US 5354668 A
TITLE: Methods for the isothermal amplification of nucleic acid molecules

Detailed Description Paragraph Right (19):
Experiments with mutant loxP sites in which either the left or right inverted repeat had been removed, has revealed that Cre is
capable of binding to partial loxP sites, but is incapable of mediating efficient recombination between such sites. Insertions in
the spacer region impair the ability of Cre to catalyze recombination. Of particular interest to the present invention is the use
of a loxP511 mutant site.

09/648 790
Jul 1 A6

=> s recombinase? or transpos? or integrase?
L1 99692 RECOMBINASE? OR TRANSPOS? OR INTEGRASE?

=> s flank? or adjacent
L2 818953 FLANK? OR ADJACENT

=> s multiple
L3 1384676 MULTIPLE

=> s site

L4 1490490 SITE

=> s l2(5n)l4
L5 15151 L2(5N) L4

=> s l3(5n)l4
L6 7467 L3(5N) L4

=> s l5 or l6
L7 22391 L5 OR L6

=> s different or alternate
L8 5519265 DIFFERENT OR ALTERNATE

=> s l1 and l7 and l8
L9 173 L1 AND L7 AND L8

=> s site?
L10 2420764 SITE?

=> s l2(5n)l10
L11 27873 L2(5N) L10

=> s l3(5n)l10
L12 35824 L3(5N) L10

=> s l11 or l12
L13 62561 L11 OR L12

=> s l1 and l8 and l13
L14 406 L1 AND L8 AND L13

=> s l14 and py<1997
1 FILES SEARCHED...
2 FILES SEARCHED...
4 FILES SEARCHED...
L15 238 L14 AND PY<1997

=> dup rem l15

PROCESSING COMPLETED FOR L15
L16 94 DUP REM L15 (144 DUPLICATES REMOVED)

=> d l16 ibib abs 1-94

L16 ANSWER 1 OF 94 MEDLINE
ACCESSION NUMBER: 96355537 MEDLINE
DOCUMENT NUMBER: 96355537 PubMed ID: 8702947
TITLE: Rho-dependent termination of transcription is governed primarily by the upstream Rho utilization (rut) sequences of a terminator.
AUTHOR: Richardson L V; Richardson J P
CORPORATE SOURCE: Department of Chemistry, Indiana University, Bloomington, Indiana 47405, USA.
CONTRACT NUMBER: AI10142 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, ***(1996 Aug 30)***
271 (35) 21597-603.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961022
Last Updated on STN: 20000303
Entered Medline: 19961010
AB A Rho-dependent transcription terminator in Escherichia coli DNA consists
of an upstream part for Rho utilization (rut) and the transcription stop point (tsp) region. To test the role of the tsp region variants of the coliphage lambda cro gene terminator, tR1, containing inserts of non-terminator sequences between its rut and tsp regions were tested for termination function. The results showed that termination occurred with high efficiency at ***multiple*** ***sites*** in each of the new sequences with the positions of the sites coinciding with transcriptional pause points in the insert sequence and that the efficiency of termination was not directly proportional to the extent of pausing at those points. Thus, in contrast to the rut sequences, which are relatively rare in DNA, many ***different*** sequence segments can function as a tsp region. Studies with isolated transcripts showed that a rut element and sequences 3' of the rut element were both needed to activate ATP hydrolysis by Rho factor with the degree of activation depending on the length and the specific sequence of the 3' segment. These results support models for Rho action in which ATP hydrolysis is coupled to interactions of Rho protein with RNA 3' of the rut region.

L16 ANSWER 2 OF 94 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 1996:332298 BIOSIS
DOCUMENT NUMBER: PREV199699054654
TITLE: Efficient in vivo manipulation of mouse genomic sequences at the zygote stage.
AUTHOR(S): Lakso, Merja; Pichel, Jose G.; Gorman, James R.; Sauer, Brian; Okamoto, Yo; Lee, Eric; Alt, Frederick W.; Westphal, Heiner (1)
CORPORATE SOURCE: (1) Natl. Inst. Health, Build. 6B, Room 413, 6 Center Dr., Bethesda, MD 20892-2790 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 12, pp. 5860-5865.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We describe a transgenic mouse line carrying the cre transgene under the
control of the adenovirus EIIa promoter that targets expression of the Cre ***recombinase*** to the early mouse embryo. To assess the ability of this ***recombinase*** to excise loxP-flanked DNA sequences at early stages of development, we bred EIIa-cre transgenic mice to two ***different*** mouse lines carrying loxP-flanked target sequences: (i) a strain with a single gene-targeted neomycin resistance gene ***flanked*** by loxP ***sites*** and (ii) a transgenic line carrying ***multiple*** transgene copies with internal loxP ***sites*** . Mating either of these loxP-carrying mouse lines to EIIa-cre mice resulted in first generation progeny in which the loxP-flanked sequences had been efficiently deleted from all tissues tested, including the germ cells. Interbreeding of these first generation progeny resulted in efficient germ-line transmission of the deletion to subsequent generations. These results demonstrate a method by which loxP-flanked DNA sequences can be efficiently deleted in the early mouse embryo. Potential applications of this approach are discussed, including reduction of multicopy transgene loci to produce single-copy transgenic lines and introduction of a variety of subtle mutations into the germ line.

L16 ANSWER 3 OF 94 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 96374619 EMBASE
DOCUMENT NUMBER: 1996374619
TITLE: Multiplex Cre/lox recombination permits selective site-specific DNA targeting to both a natural and an engineered site in the yeast genome.
AUTHOR: Sauer B.
CORPORATE SOURCE: National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Disease,Bethesda, MD 20892-1800, United States
SOURCE: Nucleic Acids Research, (1996) 24/23 (4608-4613).
ISSN: 0305-1048 CODEN: NARHAD
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology